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CITATION:

Ito, Takeo ...[et al]. Site-specific DNA oxidation by a dinuclear copper complex containing a photoisomerizable azobenzene ligand. *Inorganica Chimica Acta* 2013, 408: 230-234

ISSUE DATE:

2013-11

URL:

<http://hdl.handle.net/2433/179479>

RIGHT:

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Site-Specific DNA oxidation by a Dinuclear Copper Complex

Containing a Photoisomerizable Azobenzene Ligand

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Abstract

A dinuclear copper complex possessing an azobenzene backbone ($\text{Cu}^{\text{II}}_2 \mathbf{1}$) was synthesized and its affinity towards DNA was investigated and found to be dependent on the *trans-cis* isomeric forms. Upon exposure to UV light at 365 nm the *trans* form of the complex (Cu^{II}_2 *trans*- $\mathbf{1}$) underwent photoisomerization into the *cis*-isomer (Cu^{II}_2 *cis*- $\mathbf{1}$), which reverted to the original *trans*-form on exposure to visible light at 420 nm. Both the *trans* and *cis* isomers exhibited moderate DNA cleavage activity toward plasmid duplex DNA in the presence of a reducing agent 3-mercaptopropionic acid (MPA) suggesting that the Cu^{I} species could activate molecular oxygen to form a

reactive oxygen species *in situ*. Interestingly, the isomeric copper complexes showed different site-specificities in the oxidation of an oligodeoxynucleotide having single-strand–double-helix junctions in the sequence. The *trans* isomer Cu^I₂ *trans*-**1** induced oxidation at the consecutive deoxyguanosine site (GG) near the junction; on the other hand, such site-specificity was not observed in the case of Cu^I₂ *cis*-**1**.

Keywords: Multinuclear copper complex; Photoisomerization; H₂O₂; DNA oxidation; Azobenzene

1. Introduction

Transition metal complexes capable of interacting with DNA have been developed as artificial nucleases for determining the structure and dynamics of DNA and its assembly with proteins [1-5]. Some multinuclear copper complexes show high nucleobase affinity and structure-dependent DNA cleavage activity via the formation of a reactive oxygen species. Researchers have previously demonstrated that multinuclear copper (II) complexes promote efficient oxidative DNA cleavage especially at the junction of single- and double-stranded DNA in the presence of reducing agents (e.g., 3-mercaptopropionic acid [MPA]) and dioxygen [6-11]. The present study was inspired by interesting findings of earlier studies, which showed that the ligand structure and the distance between the copper centers of the complexes could alter the mode of dioxygen activation and site-specificity of the DNA cleavage [11,12], leading to the design and synthesis of a dinuclear copper complex. The conformation of the ligand and the distance between two copper centers were reversibly altered by photo-irradiation, and its DNA oxidation activity was evaluated as a new type of metallonuclease. Recently, Hirota and co-workers have developed structurally similar dinuclear metal complexes and investigated hydrolysis of DNA duplex by the isomeric forms of the complexes, and have found that only cis-forms induce hydrolysis of DNA [13,14]. They also attempted redox cleavage of DNA by employing dinuclear copper-dipeptides complexes containing azobenzene structures, but failed to observe conformation dependent activity toward duplex DNA [14]. We thus focused our attention on the copper-catalyzed activation of molecular oxygens by our developed dinuclear copper complexes and studied structure-dependent DNA oxidation as described below.

2. Experimental

2.1. General methods

Oligodeoxynucleotides ODN 1 and 2 were purchased from Life Technologies. NMR spectra were recorded on a JEOL EX 400 spectrometer and chemical shifts were expressed in ppm relative to the residual signals of the solvents. Fast atom bombardment (FAB) mass spectrometry was performed on a JEOL JMS-SX 102A mass spectrometer using nitrobenzyl alcohol as a matrix. UV–visible spectra were obtained using a JASCO V-530 UV–Vis spectrophotometer. Monochromatic light of 365 or 420 nm was obtained from a LAX-101 (100 W) light source (Asahi Spectra) with appropriate filters.

2.2. Synthesis of Azobenzene ligand **1**

N,N-Bis[2-(2-pyridyl)ethyl]-2-(4-nitrophenyl)ethylamine (**2**)^[15].

To 2-(4-nitrophenyl)ethylamine hydrochloride (Tokyo Chemical Industry, 1.12g, 5.54 mmol) in 10 ml of H₂O was added sodium hydroxide (5 M) dropwise until pH became ~9. The resulting mixture was extracted with chloroform. The organic layer was washed with brine, dried over magnesium sulfate, filtered, and then evaporated. Residual brown solid was dissolved in 5 ml of anhydrous methanol, and stirred in an atmosphere of nitrogen at 80 °C. After the addition of acetic acid (1.00 ml, 18.80 mmol) and 2-vinylpyridine (1.00 ml, 11.28 mmol), the mixture was refluxed for 8 h under N₂ atmosphere. The resulting mixture was then poured into water and extracted with Et₂O. Organic layer was washed with brine, dried over magnesium sulfate, filtered, and then dried under reduced pressure. Silica gel chromatography (10:1 chloroform/methanol) gave **2** (1.28 g, 50.8%) as a dark brown oil: ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 8.50 (d, *J* = 3.9 Hz, 2H), 8.02 (d, *J* = 8.8 Hz, 2H), 7.50 (m, 2H), 7.14 (d, *J* = 8.8 Hz, 2H), 7.08 (t, *J* = 11.5 Hz, 2H), 6.96 (d, *J* = 7.8 Hz, 2H), 2.97 (m, 6H), 2.78 (m, 6H); ¹³C

NMR (CDCl₃, 100 MHz) δ (ppm) 148.7, 148.2, 146.0, 136.1, 129.3, 123.3, 123.0, 121.0, 54.6, 53.2, 35.1, 33.0;

HRMS calcd. for C₂₂H₂₅O₂N₄ [(M+H)⁺] 377.1978, found 377.1985.

Ligand *trans*-**1**

A suspension of LiAlH₄ (1.00 g) in 30 ml of anhydrous Et₂O was vigorously stirred in an atmosphere of argon at -78 °C. To this suspension was added **2** in 30 ml of anhydrous Et₂O dropwise. The mixture was allowed to warm to room temperature and then further stirred for 3 h. The reaction was quenched slowly and successively with ethyl acetate, methanol, and H₂O with vigorously stirring. The mixture was extracted with Et₂O. Organic layer was washed with brine, dried over magnesium sulfate, filtered, and then concentrated under reduced pressure. The crude product was purified by silica gel chromatography (10:1 chloroform/methanol) to give *trans*-**1** (1.08 g, 92.1%) as an orange oil: ¹H NMR (CDCl₃, 400 MHz) δ (ppm) 8.45 (d, J = 3.9 Hz, 4H), 7.94 (d, J = 8.5 Hz, 4H), 7.45 (m, 4H), 7.09 (d, J = 8.6 Hz, 4H), 7.03 (t, J = 10.9 Hz, 4H), 6.91 (d, J = 7.6 Hz, 4H), 2.92 (m, 12H), 2.73 (m, 12H); ¹³C NMR (CDCl₃, 100 MHz) δ (ppm) 149.2, 148.2, 144.0, 138.1, 132.3, 126.3, 125.0, 119.0, 55.4, 54.3, 34.1, 32.0; HRMS calcd. for C₄₄H₄₉N₈ [(M+H)⁺] 689.4081, found 689.4091.

Preparation of [Cu^{II}₂ *trans*-**1**(ClO₄)₄•H₂O]

Copper (II) perchlorate hexahydrate (0.23 g, 0.36 mmol) was dissolved in 1 ml of THF and stirred at room temperature. To this solution was added *trans*-**1** (0.12 g, 0.18 mmol) in 1 ml of MeOH dropwise. Obtained solid was further recrystallized from methanol to give the desired product as green solid (60 mg, 0.05 mmol, 28%): *Anal.* Calc. for C₄₄H₅₀C₁₄Cu₂N₈O₁₇: C, 42.90; H, 4.09; N, 9.10. Found: C, 42.91; H, 3.97; N, 9.14%. HRMS calcd. for C₄₄H₄₉N₈Cu₂ [(M+H)⁺] 815.2667, found 815.2665.

2.3. Oxidative Cleavage of Oligodeoxynucleotides

Oligodeoxynucleotide ODN 1 or 2 (50 pmol) was radiolabeled with [γ - 32 P]-ATP (Perkin Elmer, 370 MBq/ml) and T4 Polynucleotide Kinase (Nippon Gene). ODN 1 or 2 (1 μ M) containing appropriate amount of the 32 P-Labeled ODN was annealed in cacodylate buffer (10 mM sodium cacodylate, 100 mM NaCl, pH 7.0) by heating to 90 °C, followed by slow cooling to room temperature. Cis-form complex (Cu^{II}_2 *cis*-1) rich solution was prepared by photo-illumination (365 nm) to the stock aqueous solution of Cu^{II}_2 *trans*-1 until the absorption spectra indicate the photo-stationary states. The above prepared ODN solution was incubated with various concentrations of copper complex (*cis* or *trans* form) and 3-mercaptopropionic acid (MPA, 1 mM) (total 20 μ L) at 37°C. Reaction was quenched by adding diethyl dithiocarbamic acid (10 mM) [6-11]. DNA products were precipitated by adding 10 μ L of herring sperm DNA (1 mg/ml), 5 μ L of 3 M sodium acetate (pH 5.2) and 400 μ L of cold ethanol, followed by chilling at -20 °C. The precipitated DNA was dissolved in 10 vol% piperidine, heated at 90 °C for 20 min, and then dried under reduced pressure. The dried DNA pellets were resuspended in loading buffer (8 M urea, 40% sucrose, 0.025% xylene cyanol, 0.025% bromophenol blue). The samples (1 μ L, (10-40) $\times 10^3$ cpm) were loaded onto a 20% polyacrylamide (acrylamide-bisacrylamide 19:1) gel containing 7 M urea, electrophoresed at 70 W, transferred to a cassette, and stored at -80 °C with Fuji X-ray films (RX-U). Cleavage of the labelled strand was quantified by autoradiography using the ATTO Densitograph Software (version 3.0).

2.4. Plasmid DNA Cleavage

Duplex DNA cleavage by copper complexes was performed by using supercoiled circular pUC19 Form I DNA (Toyobo). pUC19 (40.0 μ M/bp) in Tris-HCl-NaCl buffer (100 mM, pH7.4) was incubated in the presence of 3-mercaptopropionic acid (1 mM) and 0-50 μ M of the complex (total volume 10 μ L), then the products were quantified by agarose gel electrophoresis (0.8% agarose gel, ethidium bromide stain). Single strand DNA cleavage was observed as the formation of relaxed circular DNA (Form II) band. For the quenching assay, NaN_3 (4 mM), dimethylsulfoxide (4 mM), *D*-mannitol (4 mM), *t*-butyl alcohol (4 mM), superoxide dismutase (10 or 20 U), and catalase (10 or 20 U) were used as quenchers (concentrations in parentheses indicate final concentrations). The gels were visualized on a UV transilluminator ($\lambda_{\text{ex}} = 312$ nm, ETX-35.M, Vilber-Lourmat).

2.5. DNA-Binding Assay

UV-visible absorption spectra of the copper complex (50 μ M) in Tris-HCl-NaCl buffer (5 mM, pH 7.4) were measured under various concentrations of poly dG/poly dC (SIGMA-Aldrich). The intrinsic binding constants K_b between the complexes and DNA were calculated by the absorption spectral titration data ($\lambda = 420$ nm) using equation 1.

3. Results and discussion

3.1. Synthesis

An azobenzene structure was chosen for regulating the conformation of the dinuclear copper ligand (*trans*-**1**) in a reversible manner following illumination with UV light (Fig. 1). A dinuclear Zn^{II} complex possessing a similar ligand structure has been recently reported, in which the distance between two benzylic carbons of azobenzene moiety was determined to be ~12 Å [13]. The ligand *trans*-**1** was synthesized from 2-(4-nitrophenyl)ethylamine and 2-vinylpyridine in 2 steps (yield 47%, Scheme 1). Photoinduced isomerization of *trans*-**1** into the *cis*-form (*cis*-**1**) in methanol could be monitored by ¹H-NMR (Supplementary data, Fig. S1).

Subsequently, *trans*-**1** in methanol was treated with Cu^{II}(ClO₄)₂•6H₂O to afford the dinuclear complex Cu^{II}₂ *trans*-**1**. The copper complex Cu^{II}₂ *trans*-**1** underwent *trans*–*cis* isomerization upon UV irradiation at 365 nm and reached the *trans*–*cis* photostationary state within 5 min as observed from the change in absorption spectra (Fig. 2). Further exposure of the Cu^{II}₂ *cis*-**1**-rich solution to visible light at 420 nm induced *cis*–*trans* isomerization to yield the *trans* isomer.

(Fig.1)

(Scheme 1)

(Fig. 2)

3.2. DNA cleavage activity

The DNA cleavage activity of the complex was investigated and its sequence specificity

was determined by designing and synthesizing oligodeoxynucleotides (ODNs 1 and 2) that form single-strand–double helix junctions and hairpin structures (Fig. 3A). Sequential deoxyguanosine (GG) sites have been inserted in the middle of the helices (ODNs 1 and 2) and at the junctions (ODN 2) to investigate potential interaction between GG-sites and copper complexes. Non-irradiated Cu^{II}_2 *trans*-1 complex or its isomer that is formed after exposure to UV (Cu^{II}_2 *cis*-1-rich) (100 μM each) was incubated with 5'- ^{32}P -labeled ODN 1 or ODN 2 (1 μM) at 37°C in the presence of MPA (1 mM), which was used as an *in situ* reducing agent for Cu^{II} . Then, the samples were treated with hot piperidine to obtain DNA fragments that were digested at oxidatively damaged sites. In the case of ODN 1, non–site-selective weak cleavage bands were detected using polyacrylamide gel electrophoresis (Supplementary data, Fig. S2); on the other hand, in case of ODN 2, which possessed GG sites at the helix-coil junctions, site-specific DNA oxidation was observed especially at the $\text{G}_{14}\text{G}_{15}$ site near the junction (Fig. 3B[a]). Such site-specific oxidation of DNA bases at the helix-coil junction by multi-nuclear copper complexes have been previously reported [6-11]. This specificity disappeared on employing Cu^{II}_2 *cis*-1 rich fraction for the reaction (Fig. 3B[b]), demonstrating the regulation of site-specificities by photoinduced *trans*-*cis* isomerization of the complex. Interestingly, strand cleavage was also observed at nucleobases adjacent to the GG sites (C_3 and T_8). Similar specificities have been previously observed for the trinuclear copper complex, which on incubation with oligodeoxynucleotides possessing helix-coil junctions showed site-specific DNA oxidation at

nucleobases near the GG sites, possibly due to interaction between the copper complex and the GG near the DNA junction [9]. It is thus likely that photoinduced isomerization of the ligand **1** affects the accessibility of the complexes toward DNA.

(Fig. 3)

Both isomeric copper complexes showed similar weak strand-cleavage activity towards the double-helix moiety. Supercoiled circular plasmid DNA pUC19 (40 μ M/bp) was incubated with either *trans* or *cis* complex (10–50 μ M) at 37°C for 30 min, and the products were analyzed using agarose gel electrophoresis. As shown in Fig. 4, nicked circular DNA (Form II) was generated on completion of the reaction in a concentration dependent manner; however, similar DNA-cleavage activity was observed between the isomers.

3.3 DNA binding ability

The UV-visible spectra were used to determine the binding affinity of the complexes with poly dG/poly dC duplex DNA. The electronic absorption spectra of both the complexes with varying concentrations of DNA have been displayed in Fig. 5. The results of titration showed relatively low hypochromic change at around 430 nm and the binding constants K_b of $(4.7 \pm 0.6) \times 10^6 \text{ M}^{-1}$ for $\text{Cu}^{\text{II}}_{2 \text{ trans-1}}$ complex and $(3.6 \pm 0.8) \times 10^6 \text{ M}^{-1}$ for $\text{Cu}^{\text{II}}_{2 \text{ cis-1}}$ were determined from the plot of $[\text{DNA}]/(\epsilon_a - \epsilon_f)$ vs. $[\text{DNA}]$ using equation 1 [16],

$$[\text{DNA}]/(\epsilon_a - \epsilon_f) = [\text{DNA}]/(\epsilon_b - \epsilon_f) + 1/K_b (\epsilon_b - \epsilon_f) \quad (1)$$

where ε_a , ε_f and ε_b correspond to $A_{\text{obsd}}/[\text{Copper complex}]$, the extinction coefficient for the free copper complex and the extinction coefficient for the copper complex in the fully bound form, respectively. The calculated K_b values indicate that the DNA-binding affinities of the complexes are similar and small. These results are consistent with the low DNA-cleavage activity of the complexes towards the double-helix moiety of ODN 1 and ODN 2 as discussed earlier. Thus, it is plausible that the site-specific DNA oxidation could be attributed to the relatively higher accessibility of the *trans* isomer to the helix-coil junction than the *cis* isomer.

(Fig. 4)

(Fig. 5)

3.4. DNA cleavage mechanism

Previous attempts aimed at regulating DNA cleavage activity by using of dinuclear metal complexes containing an azobenzene have successfully demonstrated that strand scission of DNA depended on the ligand structure and proceeded most likely via the hydrolysis of the phosphodiester bonds of DNA [13,14]. On the other hand, our developed complex can be activated only in the presence of a reducing agent like MPA (see Fig. 6, lanes 3 and 4), which implies that Cu^{I} complexes generated *in situ* activate molecular dioxygen to form reactive oxygen species (ROS). DNA cleavage experiments were also conducted in the presence of various ROS scavengers, such as sodium azide (NaN_3 as $^1\text{O}_2$ quencher), dimethylsulfoxide (DMSO, OH radical scavenger), *D*-mannitol (MAN, OH

radical scavenger), *tert*-butyl alcohol (BU, OH radical scavenger), superoxide dismutase (SOD, O₂ radical anion quencher), and catalase (CAT, H₂O₂ quencher). As is evident in Fig. 6, none of the scavengers NaN₃, DMSO, BU, MAN, or SOD inhibited strand scission of pUC19 DNA, and only high concentrations of CAT quenched the reaction. The activation mechanism of dioxygen that leads to DNA cleavage is unclear at present, although H₂O₂ formation from mononuclear or multinuclear copper complexes have been previously reported [1,12,17,18]. Possibly, the H₂O₂ thus formed gets further activated by Cu^I and induces DNA cleavage; however, co-operative H₂O₂ activation by the two copper centers of our dinuclear copper complex is not involved in the reaction process, as both the *cis* and *trans* isomers showed similar reactivity towards double-stranded DNA.

(Fig. 6)

Thus, we have demonstrated that two isomeric forms of our dinuclear copper complex with azobenzene ligand structures exhibit different site-specificities in the oxidative cleavage of DNA containing single-strand–double-helix junction, which can be regulated by photo-irradiation. Our developed copper complex could be useful as a metallonuclease, the target specificity of which can be modulated by redesigning ligand structures of the isomeric azobenzene.

Acknowledgment

This work was supported by a research grant from the Daiwa Securities Health Foundation.

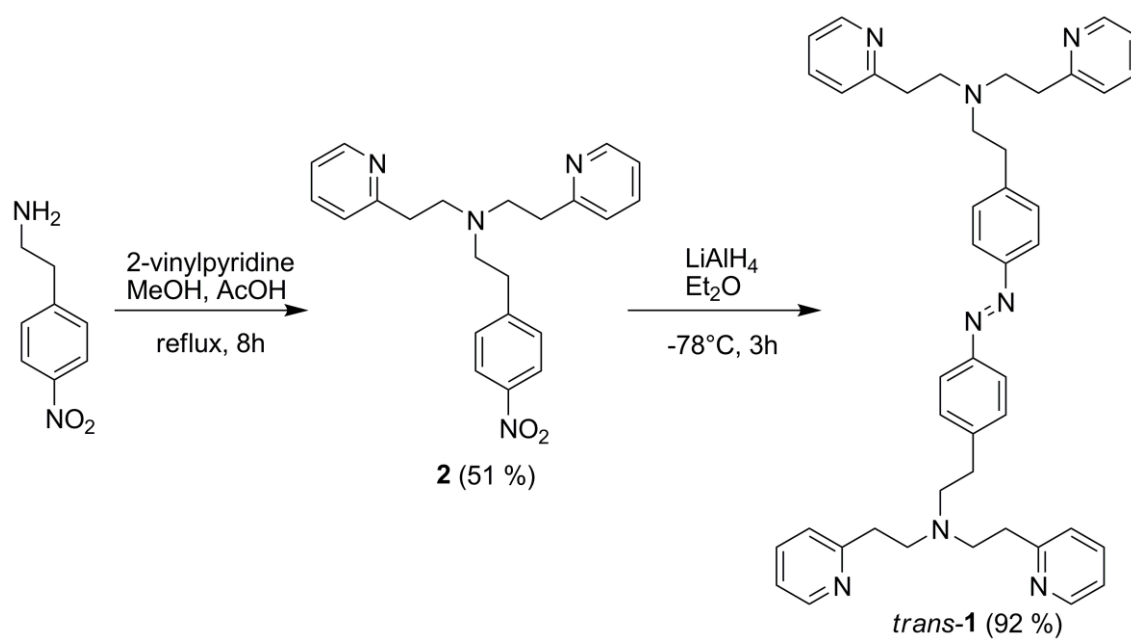
Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at **

References

- [1] C. J. Burrows, J. G. Muller, *Chem. Rev.* 98 (1998) 1109–1151.
- [2] T. A. van den Berg, B. L. Feringa, G. Roelfes, *Chem. Commun.* (2007) 180–182.
- [3] Q. Jiang, N. Xiao, P. Shi, Y. Zhu, Z. Guo, *Coord. Chem. Rev.* 251 (2007) 1951–1972.
- [4] C. Liu, L. Wang, *Dalton Trans.* (2009) 227–239.
- [5] K. L. Haas, K. J. Franz, *Chem. Rev.* 109 (2009) 4921–4960.
- [6] K. J. Humphreys, A. E. Johnson, K.D. Karlin, S. E. Rokita, *J. Biol. Inorg. Chem.* 7 (2002) 835–842.
- [7] K. J. Humphreys, K. D. Karlin, S. E. Rokita, *J. Am. Chem. Soc.* 124 (2002) 6009–6019.
- [8] L. Li, K. D. Karlin, S. E. Rokita, *J. Am. Chem. Soc.* 127 (2005) 520–521.
- [9] T. Ito, S. Thyagarajan, K. D. Karlin, S. E. Rokita, *Chem. Commun.* (2005) 4812–4814.
- [10] L. Li, N. N. Murthy, J. Telser, L. N. Zakharov, G. P. A. Yap, A. L. Rheingold, K. D. Karlin, S. E. Rokita, *Inorg. Chem.* 45 (2006) 7144–7159.
- [11] S. Thyagarajan, N. N. Murthy, A. A. Narducci Sarjeant, K. D. Karlin, S. E. Rokita, *J. Am. Chem. Soc.* 128 (2006) 7003–7008.
- [12] Q. Zhu, Y. Lian, S. Thyagarajan, S. E. Rokita, K. D. Karlin, N. V. Blough, *J. Am. Chem. Soc.* 130 (2008) 6304–6305.

- [13] A. Panja, T. Matsuo, S. Nagao, S. Hirota, *Inorg. Chem.* 50 (2011) 11437–11445.
- [14] H. Prakash, A. Shodai, H. Yasui, H. Sakurai, S. Hirota, *Inorg. Chem.* 47 (2008) 5045–5047.
- [15] T. Osako, Y. Tachi, M. Doe, M. Shiro, K. Ohkubo, S. Fukuzumi, S. Itoh, *Chem. Eur. J.* 10 (2004) 237–246.
- [16] A. Wolfe, G. H. Shimer, T. Meehan, *Biochemistry* 26 (1987) 6392–6396.
- [17] S. T. Frey, H. H. J. Sun, N. N. Murthy, K. D. Karlin, *Inorg. Chim. Acta* 242 (1996) 329–338.
- [18] M. González-Álvarez, G. Alzuet, J. Borrás, M. Pitié, B. Meunier, *J. Biol. Inorg. Chem.* 8 (2003) 644–652.



Scheme 1. Synthesis of *trans*-**1**.

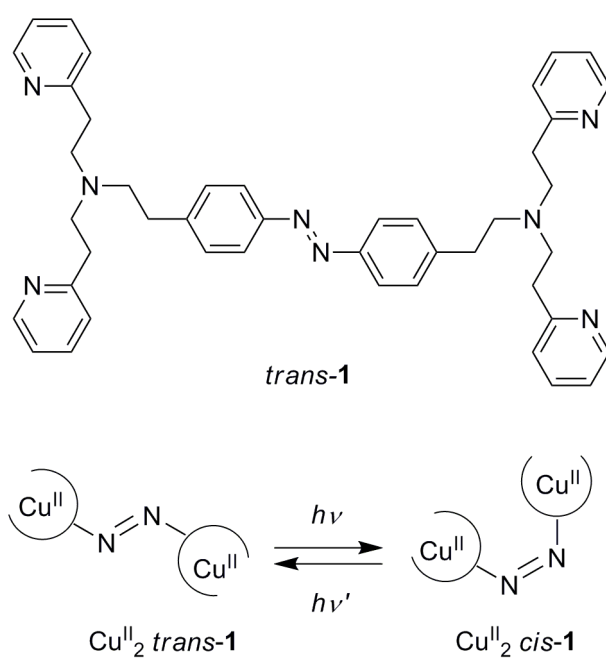


Fig. 1. Photoinduced *trans*–*cis* isomerization of Cu^{II}_2 **1**.

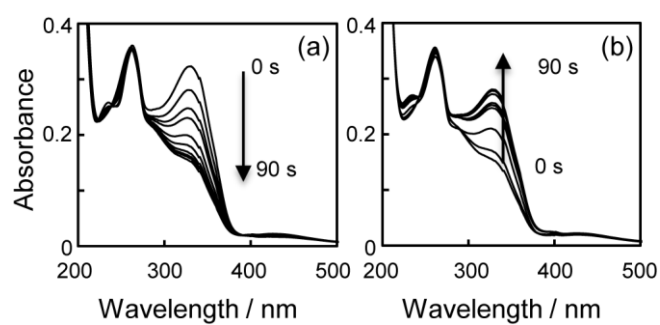


Fig. 2. Absorption spectra of Cu^{II}_2 **1** ($10\ \mu\text{M}$) in acetonitrile. (a) Cu^{II}_2 *trans*-**1** was exposed to 365-nm UV light for 0–90 s. (b) Further exposure of the solution to 420-nm visible light for 0–90 s.

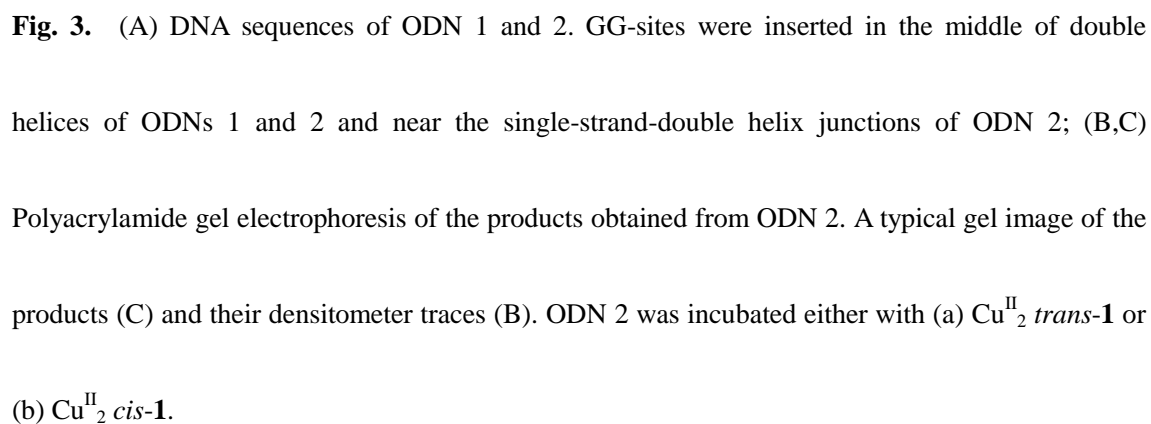


Fig. 3. (A) DNA sequences of ODN 1 and 2. GG-sites were inserted in the middle of double helices of ODNs 1 and 2 and near the single-strand-double helix junctions of ODN 2; (B,C) Polyacrylamide gel electrophoresis of the products obtained from ODN 2. A typical gel image of the products (C) and their densitometer traces (B). ODN 2 was incubated either with (a) Cu^{II}_2 *trans*-**1** or (b) Cu^{II}_2 *cis*-**1**.

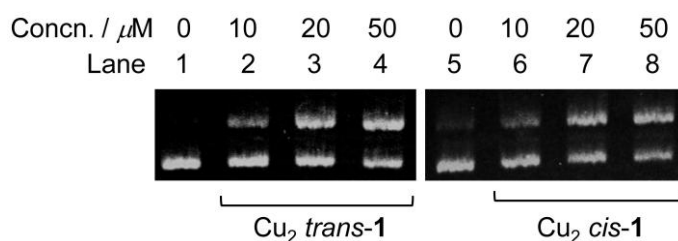


Fig.4. Agarose gel electrophoresis of DNA cleavage products. pUC19 plasmid DNA (40 μ M / bp) in Tris-HCl-NaCl buffer (100 mM, pH 7.4) was incubated with Cu^{II}_2 *trans*-1 or Cu^{II}_2 *cis*-1 (0–50 μ M) in the presence of MPA (1 mM) at 37°C for 30 min.

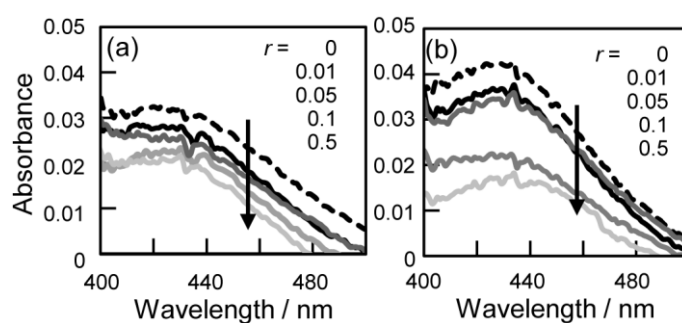


Fig. 5. Absorption spectra of (a) Cu^{II}_2 *trans*-1 and (b) Cu^{II}_2 *cis*-1 (50 μ M) in Tris-HCl-NaCl buffer (5 mM, pH 7.4) with increasing concentration of poly dG/poly dC; $r = [\text{poly dG/poly dC}]/[\text{copper complex}] = 0\text{--}0.5$.

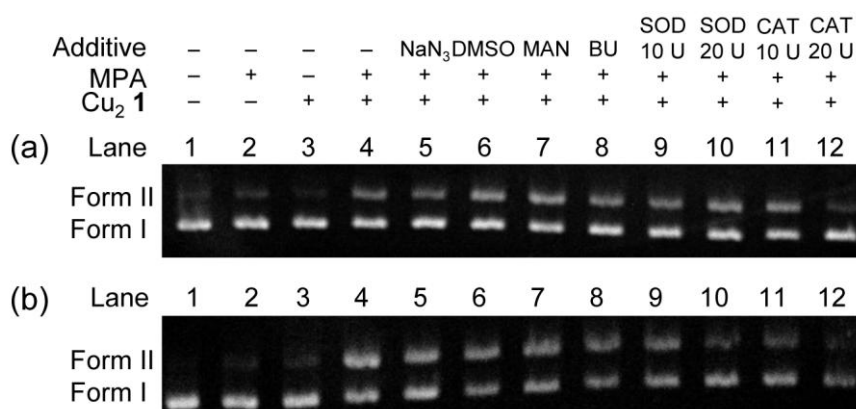


Fig. 6. Agarose gel electrophoresis of DNA cleavage products. pUC19 plasmid DNA (40 μ M/bp) in Tris-HCl-NaCl buffer (100 mM, pH 7.4) was incubated with (a) Cu^{II}₂ *trans*-1 or (b) Cu^{II}₂ *cis*-1 (20 μ M) in the presence of additives for 30 min at 37°C. Lane 1. DNA alone; lane 2, DNA + MPA; lane 3, DNA + Cu complex; lane 4, DNA + MPA + Cu complex; lane 5, DNA + MPA + Cu complex + NaN₃; lane 6, DNA + MPA + Cu complex + DMSO; lane 7, DNA + MPA + Cu complex + MAN; lane 8, DNA + MPA + Cu complex + BU; lane 9, DNA + MPA + Cu complex + SOD (10 U); lane 10, DNA + MPA + Cu complex + SOD (20 U); lane 11, DNA + MPA + Cu complex + CAT (10 U); lane 12, DNA + MPA + Cu complex + CAT (20 U).